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(54) Title: METHOD FOR ISOLATION OF SYLIMARIN FROM SYLIBUM MARIANUM SEEDS

(57) Abstract: This invention relates to a method for isolation of sylimarin from silybum Marianum seeds wherein the seeds are grinded without precooling. The follow defatting with hydrocarbon solvent, extraction with medium polarity solvent, removal of water from evaporating residue lagged after extraction, purification of dry extract, separation, washing and drying of obtained crystals.

METHOD FOR ISOLATION OF SYLIMARIN FROM SYLIBUM MARIANUM SEEDS

Sylimarin is a trivial term for a complex composition of compounds: silybin, isosilybin, silydianin, silychristin, taxifolin, and kvercetin and its the most significant natural resource are the seeds of *Silybum marianum*. Sylimarin has been proved as extremely hepatoprotective and it is significant active substance in numerous herbal drugs. The most significant sylimarin component is silybin whereas the other components are present in smaller amounts. Most of the studies from sylimarin pharmacology area relate to the activities of the whole composition whereas individual activities of sylimarin components are not well-known about. Due to this reason, the production of sylimarin is directed to as quantitative as possible isolation from the seeds of *Silybum marianum* and processing into stabile crystal-like form. Such sylimarin is suitable for production of various pharmaceutical forms in the domain of herbal medicines or dietetics.

According to the process described in patent no.: US 6 309 678, the isolation of sylimarin includes cooling the seeds to -20° C in order get it powdered afterwards. Powdered seeds are extracted by n-hexane for defatting. After this, defatted seeds are extracted with acetonitrile at room temperature. By evaporation of acetonitrile extract, a material is formed that has to be further purified by extraction with dichlormethane and the product, sylimarin, is obtained by redissolving the residue in minimal amount of acetonitrile and with precipitating it with the addition of distilled water.

Described process as a part of the document includes unacceptable precooling of the seeds to -20° C prior to defatting. Described extracting of defatted material includes consumption of about 890 ml of acetonitrile for 100 g of the seeds (ratio 1 : 8.9, m/V) which is quite unacceptable for the industrial scale. Apart from this, acetonitrile is significantly toxic. By evaporation of the acetonitrile extract, such material is obtained that obviously contains some oil and was necessarily washed with dichlormethane. Dichlormethane is a bad choice of solution for the above mentioned purpose because it is significantly toxic and its boiling point is very low, resulting in great losses on industrial scale. Purification of sylimarin

by precipitating it with water from acetonitrile solution might be problematic because it can extract a product of high purity and colour but resin-like consistency.

The process described in the document US 4 368 195, just as its numerous versions, has been examined in laboratory because it was assumed that ethylacetate was more appropriate solution than acetonitrile, due to its lower price and toxicity. The process includes defatting of the seeds by cold pressing. Such defatted residue is extracted several times (3 x) in boiling ethylacetate. Obtained ethyl acetate extract is evaporated and its residue is processed by three-component extraction of water/methanol/dichlormethane (chloroform) wherein the residual oil passes into dichlormethane fraction and sylimarin into aqueous-methanolic fraction. By evaporation of the aqueous-methanolic fraction the crude product is obtained which is further purified by precipitating it from methanolic solution, by adding distilled water.

Described process includes cold pressing of the seeds which has its technological advantages. However, substantial part of oil is left in the residue so that ethyl acetate extract contains a lot of oil, apart from sylimarin. The oil can be removed only by three-component extraction. Furthermore, the process requires application of large volume of ethyl acetate for the mass of the 3x1 : 10 m/V which is apsolutely non-economic for the industrial scale. Furthermore, three-component extraction requires application of large amount of toxic organic solutions, dichlormethane and methanol, whose regeneration level is questionable. Furthermore, sylimarin lags in aqueous-methanolic fraction whose evaporation requires relatively much energy, wherein stability of dissolved sylimarin is questionable in the final phase of evaporation when the substance is exposed to relativly high temperatures in aqueous medium, in which the solubility of oxygen from the air is by far higher than the solubility in organic solvents. Additional purification of sylimarin in way of precipitating it from methanolic solution by adding distilled water might be problematic as in the previous process because the product can be extracted in resin-like consistency.

It has been concluded that both processes are not (at least in the described form) suitable for industrial isolation of sylimarin from the seeds.

A new method for the isolation of sylimarin and oil from the seeds has been developed. The method includes grinding the seeds, extraction of oil with means for hot

defatting and extraction of defatted seeds with a medium polarity solvent. After the filtration, the extract is evaporated to dryness and the residue is azeotropic dried, with adding of means for drying. Dried extract is being defatted in hot ether wherein, after the chilling, filtrating and drying, a concentration of sylimarin in form of homogenous yellow-orange crystal-like substance is obtained. It has high melting point (ca. 140-165° C). Yield of sylimarin is 2,0-2,5% according to the content of total sylimarin of 86-97% and approx. 20% of the oil calculated by a crude seed.

In comparison with the previously described methods, the new one does not use toxic solvents, dichlormethane, methanol or acetonitrile. The described method applies acetone as the least toxic and by far the cheapest medium polarity solvent. The method is developed with application of minimal (optimum) amounts of organic solvents with regeneration rate of approx. 90-95%. All chemical and technological details are stated in description of the method.

The method comprises the following steps:

1. grinding the seeds of *Sylbum Marianum*
2. extracting the seed powder with means for defatting
3. filtrating the defatted seeds
4. extraction of defatted seeds with acetone
5. filtrating the extracted seeds
6. evaporating the acetone extract
7. azeotropic drying of the extract with toluene
8. secondary defatting the extract with diisopropyl ether
9. filtrating the sylimarin
10. drying the sylimarin

Crude seeds are powdered in mills with rotating knife with the application of screen up to 40 mesh, prior to defatting. After this, defatting is conducted with application of means for defatting, such as hydrocarbon. N-hexane is used as means for defatting in preferable version of the invention. Petrol ether is used in other version of the invention. Suspension in the applied ratio herbal material : solvent (m/V) can normally be stirred with mechanical stirrer.

The filtration is conducted in vacuum. The filtrate has intensive yellow colour and contains oil of the seeds in n-hexans. The residue is heated in vacuum to provide complete removal of extraction solvent traces and n-hexane traces. Under the stated conditions, 70° C during 2-3 hours, there are no changes in the obtained oil if we take into account quality and quantity, i.e., there is no loss of quality. According to this invention, defatted seeds do not need to be dried in order to rid them of the traces of absorbed n-hexane but to be extracted with medium polarity solvent, such as acetone, immediately after vacuuming.

Extraction with acetone is conducted at the temperature of approx. between 18° C and 56° C. Optimum extraction time is approx. from 24 to 72 hours, depending on the temperature at which the extraction is conducted. Filtration follows the extraction. The stated method provides regeneration level of acetone of approx. 95% which is, just as n-hexane in defatting phase, really being used by its 5%.

In the other version, defatting is conducted in common percolator at room temperature during at least 48 hours. In this case there is no danger of functioning at increased temperature with n-hexane: After defatting and removing the traces of n-hexane, percolation with acetone for isolation of sylimarin is continued.

Azeotropic distillation follows, by which water from the residue after evaporating the acetone filtrate is removed. During this, after evaporating the acetone, a receiving plate is replaced, wherein the mixture of toluene and water is gathered. The stated mixture is easily separated in the extractor wherein the upper, toluene fraction is stored. Under the stated conditions, 80% of the used toluene is regenerated with a part of it being wasted in the vacuum system. However, work in vacuum is necessary to provide drying at a temperature as low as possible.

For purification, i.e. removing the residual oil, according to the invention ethers are applied as means extraction. According to the invention, ethers with 4 to 8 C atoms such as tetrahydrofuran, or diisopropyl ether, or diethyl ether are appropriate for secondary defatting.

It has been found that diisopropyl ether acts as the most efficient solvent for defatting the extracts wherein the oil is completely dissolved while sylimarin constituents remain almost completely suspended. One part of the evaporated residue can stay attached to the walls of the gob and it is not removed spontaneously during heating at the temperature for the return of solvent. Therefore, a mechanical intervention is needed. On industrial scale, a

reactor with mechanical mixer whose shape follows the geometry of the dish is used. The purpose of it is to avoid the sylimarin getting glued to the walls as soon as during azeotropic drying with toluene. Once formed, suspension of sylimarin constituents is nicely defatted and easily filtered *in vacuo* after the cooling. Further cooling of the suspension do not substantially affect the level of use, since solubility of sylimarin constituents in diisopropyl ether is extremely low.

The next example serves only as an illustration of the invention and can by no means be used for defining the range and content of the invention.

The example

400.00 g of grinded seeds were weighed in a three-necked flask of 2000 ml and 1200 ml of n-hexane was poured into it (of approx. 96%, Merck, for synthesis). Obtained suspension was heated with stirring with a mechanical mixer and with a cooler from the room temperature, approx. 22-23° C, to the temperature of the return of solvent, approx. 63° C, during 30 minutes. The suspension was stirred at the temperature of the return of solvent, approx. 62-63° C, during 3 hours. After this, the mixture was cooled, herbal material sucked out through the Buchner funnel with subpressure. 2x100 ml of n-hexane was used for the removing and washing of the flask while additional 2x100 ml of n-hexane was used for washing of the sucked out herbal material. Approx. 1500 ml (about 95% of the whole amount of the used n-hexane) of n-hexane was pre-distilled and thus regenerated from the filtrate while the residual pale yellow oil was heated at 70° C in vacuum, at 8-10 mbar, during 2 hours.

Clear yellow-orange oil of weak characteristic scent was obtained:

BATCH-1 : 60.05 g (15.0%, calculated by a crude seed)

BATCH-2 : 61.41 g (15.4%, calculated by a crude seed)

BATCH-3 : 61.98 g (15.5%, calculated by a crude seed)

Defatted *Sylbum marianum* seeds (approx. 340 g) were removed into the three-necked flask of 2000 ml and 1200 ml of acetone was poured into it, and obtained suspension was being stirred with a mechanical mixer at the room temperature during 72 hours. After this, the suspended herbal material was sucked out through the Buchner funnel with subpressure. For removal of the residual herbal material, 2x100 ml of acetone was used, and 2x100 ml of acetone for

additional washing of herbal material. From the obtained yellow filtrate (approx. 1550-1580 ml), acetone was pre-distilled by distillation at the atmospheric pressure, wherein approx. 1500-1530 ml (93.8-95.6%) of the whole amount of it was regenerated.

60 ml of toluene was added to the residual and evaporated in a rotating evaporator at the temperature of 80-85 C and the pressure of 50-60 bar, wherein 25-27 g (6.25-6.75 g) of dry extract was obtained in form of yellow-orange crystals, mottled with yellow-orange oil. 50 ml of diisopropyl ether was added to the dry extract and with stirring heated to the temperature at which the solvent returns, approx. 67-69° C, during 25-30 minutes. The suspension was heated with stirring in hot diisopropyl ether during the next 30 minutes. After this, the suspension was cooled to the room temperature during approx. 1 hour. Crystals were then sucked out and washed with 2x25 ml od diisopropyl ether. The product was dried in a vacuum drier at 40° C and the pressure of 8-10 mbar, during 24 hours.

Sylimarin (1), in form of small shiny crystals, with the colour ranging from yellow to light orange, was obtained.

BATCH-1 : 8.52 g (2.13%), t. t. 142.2-165.0 C

BATCH-2 : 9.02 g (2.26%), t. t. 143.0-164.2 C

BATCH-3 : 8.91 g (2.23%), t. t. 140.2-161.1 C

From the yellow diisopropyl ether filtrate, after the filtration of sylimarin (approx. 100 ml) by distillation at the atmospheric pressure, 95-96 ml (95-96%) of diisopropyl ether was regenerated. The residue was dried in vacuum at 8-10 mbar with heating at 70° C, during 2 hours wherein secondary yellow-orange oil was obtained. The oil was to be further filtrated to be rid of traces (less than 1%) suspended in diisopropyl ether of soluble compounds from the extract.

BATCH-1 : 17.84 g (4.46%, calculated by a crude seed)

BATCH-2 : 17.23 g (4.31%, calculated by a crude seed)

BATCH-3 : 18.70 g (4.68%, calculated by a crude seed)

The total of obtained oil: (primary + secondary)

BATCH-1: 77.89 g (19.5%, calculated by a crude seed)

BATCH-2: 78.64 g (19.7%, calculated by a crude seed)

BATCH-3 : 80.86 g (20.2%, calculated by a crude seed)

Optimum conditions of drying of sylimarin have been stated. Due to its phenol components, sylimarin is sensitive to oxygen and light. The stated method enables successful drying of sylimarin because after it is sucked out, it comprises only absorbed diisopropyl ether which is very easily dried.

Diisopropyl ether, acetone and n-hexane are regenerated to approx. 95% of their use and can be used again for the same purpose. Commercial diisopropyl ether is stabilized with approx. 50 ppm of antioxidant, such as 2,6-di-*terc*-butyl-4- methylphenol (BHT), however, solvent which is regenerated after distillation do not contain a stabilizer any more. In case of frequently repeated application of the same diisopropyl ether, solvent as such can be used without problems with a short storing in well sealed barrels. For longer storing, BHT (the cheap one) is to be added to the regenerated solvent, approx. 5 g on 100 l.

Sylimarin obtained by the described method is a crystal-like matter of orange colour, without scent, and is melted at temperature range between 140 and 165° C.

In sylimarin IR-spectrum, a characteristic stretching band of alcoholic and phenol O-H bonds at 3400, and stretching band of chetonic C=O bond of about 1640 cm are visible.

IR (KBr) v: 3401 (O-H, phenol group), 2928, 1745, 1641 (C=O, keto-group), 1513, 1465, 1358, 1275, 1160, 1160, 1083, 1027, 992, 813, 782, 644 cm.

In IR spectrum of *Silybum marianum* oil the band appears at about 1744 cm. It is characteristic for stretching of esthetic C=O bond.

IR (film) v: 3009, 2926, 2855, 1744 (C=O, esthetic group), 1656, 1466, 1418, 1378, 1239, 1163, 1099, 914, 723 cm.

Qualitative (IR spectrum, thin-layer chromatography) and quantitative (spectrophotometry) analysis were conducted on the obtained sylimarin

Quantity analysis of sylimarin concentrate obtained by the described method was done in spectrophotometry way with the use of 2,4.dinitrophenylhydrazine (DNPH) as reagent, by the method described in the literature (H. Wagner, P. Diesel, M. Seitz, *Arzneim. Forsch.* (*Drug Res.*) 24 (1974) 466-471). The results are in Table 1.

Table 1. The results of quantity content of the total sylimarin in sylimarin samples, batches 1,2 and 3, obtained according to the described method.

| SAMPLE | measured weight | measured absorption | calculated | TOTAL |
|----------------------------|-----------------|---------------------|-----------------------|-----------------|
| | | | absorption per SYLIM. | 50.00 mg. subs. |
| | | | (%) ¹ | |
| Silibyn-stand ² | 0.05044 | 0.237 | 0.235 | 100 |
| Sylimarin, B-1 | 0.05043 | 0.214 | 0.212 | 90.21 |
| Sylimarin, B-2 | 0.05002 | 0.203 | 0.203 | 86.38 |
| Sylimarin, B-3 | 0.05010 | 0.227 | 0.227 | 96.60 |

¹ The content of total sylimarin presents the content of total ketone, i.e., all the sylimarin components with chetonic functionality (so called DNPH of positive compounds).

² The silibyn standard prepared by preparatory chromatography, declared as 100% sylimarin.

Literature

1. The Merck Index, 12th Edition, Merck&Co., New York (1996) 1464.
2. H. M. Rauen, H. Schriewer, *Arzneim. Forsch. (Drug Res.)* **23** (1973) 148-170.
3. R. Seeger, G. Beck, K. Gretzer, A. Heim, *Arzneim. Forsch. (Drug Res.)* **24** (1974) 868-873.
4. G. Hahn, H. D. Lehmann, M. Kurten, H. Uebel, G. Vogel, I. Baumann, I. Dobberstein, E. Eisen, A. Ersfeld, S. Kruger, E. Meier, H. Walther, *Arzneim. Forsch. (Drug Res.)* **18** (1968) 698-704.
5. H. Wagner, L. Horhammer, R. Munster, *Arzneim. Forsch. (Drug Res.)* **18** (1968) 688-696.
6. G. Halbach, W. Trost, *Arzneim. Forsch. (Drug Res.)* **24** (1974) 866-868.
7. A. Pelter, *Tetrahedron Lett.*, **25** (1968) 2911-2916.
8. L. Merlini, A. Zanarotti, A. Pelter, M. P. Rochefort, R. Hansel, *J. Chem. Soc., Perkin Trans. 1* (1980) 775-778.
9. L. Merlini, A. Zanarotti, A. Pelter, M. P. Rochefort, R. Hansel, *J. Chem. Soc., Perkin Trans. 1* (1979) 695.
10. R. Hansel, G. Schopflin, *Tetrahedron Lett.*, **37** (1967) 3645-3648.
11. Kahol et al., **US Pat. 6,309,678** (2001).
12. Madaus et al., **US Pat. 4,368,195** (1983).
13. H. Wagner, P. Diesel, M. Seitz, *Arzneim. Forsch. (Drug Res.)* **24** (1974) 466-471.
14. H. Wagner, S. Bladt, *Plant Drug Analysis*, Springer Verlag (1996) 234-235.

PATENT CLAIMS

1. Method for isolation of sylimarin from *Silybum Marianum* without precooling seeds, comprising the following steps:
 - a) grinding the seeds to a fine powder
 - b) defatting the pulverized seeds with hydrocarbon solvent
 - c) extraction of defatted seeds with medium polarity solvent at the temperature from 18 to 56° C in order to obtain sylimarin extract
 - d) evaporation of the extract from step c)
 - e) removal of water from the evaporation residue from step d)
 - f) purification of dry extract from the previous step from residual oil
 - g) separation, washing and drying of the obtained crystals
2. Method for isolation of sylimarin from *Silybum Marianum* according to the claim 1, characterized by the fact that grinding is run in a mill with rotating knives and screen of up to 40 mesh.
3. Method for isolation of sylimarin from *Silybum Marianum* seeds according to the claim 1, characterized by the fact that grinded seeds is removed into a reactor with mechanical mixer whose form is following geometry of reactor's plate.
4. Method for isolation of sylimarin from *Silybum Marianum* seeds according to the claim 3, characterized by the fact that defatting is done in an extractor at the temperature at which solvent returns.
5. Method for isolation of sylimarin from *Silybum Marianum* seeds according to the claim 1, characterized by the fact that defatting is done in percolator at the room temperature.
6. Method for isolation of sylimarin from *Silybum Marianum* seeds according to the claim 1, characterized by the fact that hexane and petrol ether are used as solvents for defatting.
7. Method for isolation of sylimarin from *Silybum Marianum* seeds according to claim 6, characterized by the fact that n-hexane is used as solvent for defatting.
8. Method for isolation of sylimarin from *Silybum Marianum* seeds according to the claim 7, characterized by the fact that ratio between the seeds and n-hexane is from 1:2 to 1:5 m/V.
9. Method for isolation of sylimarin from *Silybum Marianum* seeds according to the claim 8, characterized by the fact that ratio between the seeds and n-hexane is 1:3 m/V.

10. Method for isolation of sylimarin from *Silybum marianum* seeds according to the claim 1, characterized by the fact that acetone is used as medium polarity solvent for the extraction of defatted seeds.
11. Method for isolation of sylimarin from *Silybum marianum* seeds according to the claim 1, characterized by the fact that the extraction of defatted seeds is done in an extractor during 24 hours, at the approx. temperature at which acetone returns.
12. Method for isolation of sylimarin from *Silybum marianum* seeds according to the claim 1, characterized by the fact that the extraction of defatted seeds is done in percolator during 72 hours, at the room temperature.
13. Method for isolation of sylimarin from *Silybum marianum* seeds according to the claim 10, characterized by the fact that ratio between the seeds and acetone is from 1:2 to 1:5 m/V.
14. Method for isolation of sylimarin from *Silybum marianum* seeds according to the claim 13, characterized by the fact that ratio between the seeds and acetone is 1:3 m/V.
15. Method for isolation of sylimarin from *Silybum marianum* seeds according to the claim 1, characterized by the fact that evaporated acetone residue is dried by azeotropic distillation with toluene.
16. Method for isolation of sylimarin from *Silybum marianum* seeds according to the claim 1, characterized by the fact that purification of dry extract from claim the 15 is done by ethers with 4 to 8 C atoms.
17. Method for isolation of sylimarin from *Silybum marianum* seeds according to the claim 16, characterized by the fact that purification of sylimarin is done approx. at the melting temperature of used ether.
18. Method for isolation of sylimarin from *Silybum marianum* seeds according to the claim 16, characterized by the fact that purification of dry extract is done with tetrahydrofuran, diisopropyl ether or diethyl ether.
19. Method for isolation of sylimarin from *Silybum marianum* seeds according to the claim 18, characterized by the fact that purification of dry extract is done with diisopropyl ether at the temperature of approx. 67 to 69 C, during 25 to about 30 minutes.
20. Method for isolation of sylimarin from *Silybum marianum* seeds according to the claim 1, characterized by the fact that suspension is cooled to the room temperature, during 1 hour.
21. Method for isolation of sylimarin from *Silybum marianum* seeds according to the claim 1, characterized by the fact that crystals of purified sylimarin are filtered and several times washed with diisopropyl ether.

22. Method for isolation of sylimarin from *Silybum marianum* seeds according to the claim 1, characterized by the fact that product is dried in drying section.
23. Method for isolation of sylimarin from *Silybum marianum* seeds according to the claim 22, characterized by the fact that product is dried in drying section at increased temperature and decreased pressure.
24. Method for isolation of sylimarin from *Silybum marianum* seeds according to the claim 17, characterized by the fact that product is dried in drying section at 40° C and the pressure of 8 to about 10 mbar.

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

WPI Data, PAJ, EPO-Internal, BIOSIS, EMBASE, PASCAL, CAB Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|----------|---|-----------------------|
| A | US 6 309 678 B1 (TANDON SUDEEP ET AL) 30 October 2001 (2001-10-30) cited in the application ---- | |
| A | US 4 368 195 A (MADAUS ROLF ET AL) 11 January 1983 (1983-01-11) cited in the application ---- | |

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Patent family members are listed in annex.

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INTERNATIONAL SEARCH REPORT

Information on patent family members

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|--|------------------|-------------------------|----------|---|
| US 6309678 | B1 | 30-10-2001 | DE SU | 10016449 A1 2914330 A1 1433391 A3 |
| US 4368195 | A | 11-01-1983 | DE | 30-10-1980 23-10-1988 |